

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 021-2 (98102wo)	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 99/ 04317	International filing date (day/month/year) 16/12/1999	(Earliest) Priority Date (day/month/year) 21/12/1998
Applicant ADVANTA TECHNOLOGY LIMITED ET AL		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1
☐ None of the figures.

PC-A5B 99/04317

IPC 7 C12N15/82 C12N5/10 A01H5/00

IPC 7 C12N A01H

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AN YONG-QIANG ET AL: "Strong, constitutive expression of the Arabidopsis ACT2/ACT8 actin subclass in vegetative tissues." PLANT JOURNAL 1996, vol. 10, no. 1, 1996, pages 107-121, XP000876842 ISSN: 0960-7412	16
Y	cited in the application the whole document	1-4, 8-10, 16, 17, 19
Y	WO 97 32011 A (CIBA GEIGY AG ;VOLRATH SANDRA L (US); JOHNSON MARIE A (US); POTTER) 4 September 1997 (1997-09-04) pages 32, 81, 37	1-4, 8-10, 16, 17, 19
	—/ —	

☒ Patent family members are listed in annex.

"g" document member of the same patent family

Holtorf, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 14824 A (ICI PLC) 3 September 1992 (1992-09-03) cited in the application the whole document	12, 15, 17-20
E	WO 00 20571 A (SAAD MOHAMMED EID ; AGRICULTURAL GENETIC ENGINEERING (EG); PIONEER HI) 13 April 2000 (2000-04-13) see page 24, 25 the whole document	1, 4, 8-11, 16, 17, 19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/04317

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9732011	A	04-09-1997	AU 1984697 A	16-09-1997
			AU 2065497 A	16-09-1997
			BR 9707769 A	27-07-1999
			BR 9707783 A	27-07-1999
			CA 2247074 A	04-09-1997
			CA 2247797 A	04-09-1997
			CN 1212724 A	31-03-1999
			CN 1212725 A	31-03-1999
			CZ 9802726 A	16-12-1998
			CZ 9802727 A	11-11-1998
			EP 0883682 A	16-12-1998
			EP 0885305 A	23-12-1998
			HU 9900623 A	28-06-1999
			HU 9901044 A	28-07-1999
			PL 328617 A	01-02-1999
			PL 328651 A	15-02-1999
			WO 9732028 A	04-09-1997
			US 5939602 A	17-08-1999
WO 9214824	A	03-09-1992	AU 656761 B	16-02-1995
			AU 1207492 A	15-09-1992
			BG 61614 B	30-01-1998
			BG 98069 A	30-06-1994
			BR 9205664 A	07-06-1994
			CA 2107804 A	26-08-1992
			EP 0636181 A	01-02-1995
			FI 933718 A	24-08-1993
			HU 67049 A, B	30-01-1995
			JP 6504914 T	09-06-1994
			RO 114979 A	30-09-1999
			US 5866778 A	02-02-1999
WO 0020571	A	13-04-2000	NONE	

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P.02

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference DW/RM/98102WO		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/418) FOR FURTHER ACTION	
International application No. PCT/GB98/04317	International filing date (day/month/year) 18/12/1999	Priority date (day/month/year) 21/12/1998	
International Patent Classification (IPC) or national classification and IPC C12N15/82			
Applicant ADIVANTA TECHNOLOGY LIMITED et al.			
<p>1. This International preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.18 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 3 sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 			
Date of submission of the demand 16/06/2000		Date of completion of this report 09.04.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80299 Munich Tel. +49 89 2399-0 Tx: 523656 opmu d Fax: +49 89 2399-4465		Authorized officer Vix, O Telephone No. +49 89 2399 7326 	

Form PCT/IPEA/409 (cover sheet) (January 1994)

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/04317

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
- Description, pages:

1-11 as originally filed

Claims, No.:

1-21 as received on 27/12/2000 with letter of 22/12/2000

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b));
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/04317

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(o)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Yes:	Claims 1-15, 17-21
	No:	Claims 16
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-21
Industrial applicability (IA)	Yes:	Claims 1-21
	No:	Claims

**2 Citations and explanations
see separate sheet****VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/04317

Reference is made to the following documents:

- D1: AN YONG-QIANG ET AL: 'Strong, constitutive expression of the Arabidopsis ACT2/ACT8 actin subclass in vegetative tissues.' PLANT JOURNAL 1996, vol. 10, no. 1, 1996, pages 107-121, XP000876842 ISSN: 0960-7412 cited in the application
- D2: WO 97 32011 A (CIBA GEIGY AG ;VOLRATH SANDRA L (US); JOHNSON MARIE A (US); POTTER) 4 September 1997 (1997-09-04)
- D3: WO 92 14824 A (ICI PLC) 3 September 1992 (1992-09-03) cited in the application
- D4: WO 00 20571 A (SAAD MOHAMMED EID ;AGRICULTURAL GENETIC ENGINEERI (EG); PIONEER HI) 13 April 2000 (2000-04-13)

Re Item V**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The application relates to methods of producing a genetically modified *Compositae* plant by transformation of a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin 2 gene promoter from *Arabidopsis thaliana*.

1. Novelty (Art. 33(2) PCT)

- 1.1 Priority documents have not been available at the time of establishing this preliminary opinion. The opinion has been established under the assumption of valid priority rights. Should this however not be the case, the document D4 cited in the ISR as E-document might become important if its own priority date is valid.
- 1.2 D1 describes a vector comprising the ACT2 sequence fused with the beta-glucuronidase reporter gene (*Gus*) that is used to monitor the constitutive expression in different vegetative tissue (D1 page 111 and 119). As such, this construct in D1 takes away the novelty of claim 16 which relates to a vector including a *Gus* DNA sequence under the control of ACT2 gene promoter.

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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/04317

2. Inventive step (Art. 33(3) PCT)

Claims 1-8 refer to a method of producing a genetically-modified *Compositae* plant using an heterologous DNA sequence under the control of the actin 2 (ACT2) gene promoter. Claim 18 deals with the vector used in the process of claim 1.

The claims 9-15 are directed towards the genetically modified *Compositae* plant cells obtained by the process of claim 1, whereas claims 17-21 relate to the *Compositae* plants comprising those cells.

The closest prior art D2 discloses methods for engineering plants with DNA encoding protox (protoporphyrinogen oxidase) enzymes or mutants thereof. Examples of transgenic plants transformed with recombinant vectors (cited in D2 page 31) comprising protox gene coupled with an active promoter are disclosed in D2. The *Arabidopsis* actin promoter is clearly cited in D2 (page 37) as a promoter capable of functioning in plants or plant cells, clearly indicating to the skilled person a possible use of such a promoter in specific plant vectors. Moreover, diverse plants of agronomical interest are cited in page 32 of D2, and a *Compositae* plant (in this case sunflower) is cited among the most preferred plants. In consequence, a method for producing a transgenic sunflower using the transformation with a recombinant DNA vector comprising the actin promoter is clearly suggested in D2, although not disclosed specifically in the examples (choice in a list of plants and a list of possible promoters).

The problem to be solved by the present invention may therefore be regarded as a method for obtaining genetically-modified *Compositae* plant cells comprising a transformation of the plant with an heterologous DNA construct under the control of a promoter capable of an strong and effective expression in said plant.

From D1, it is known that the ACT2 gene promoter of *Arabidopsis* is a strong and constitutive actin gene promoter. In page 111 of D1, the construct comprising the ACT2 promoter coupled with the *Gus* reporter gene showed a strong expression in all vegetative tissues of the plant. Obviously the ACT2 promoter is an interesting candidate for strong recombinant expression in plants.

Therefore, the skilled person interested in solving the technical problem would combine the teaching of D2 (possibility to choose the sunflower for the transformation

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INTERNATIONAL PRELIMINARY

International application No. PCT/GB99/04317

EXAMINATION REPORT - SEPARATE SHEET

using a recombinant DNA vector comprising a promoter such as Actin) with the technical features of D1 describing in details the technical features of the Actin promoter. Thus, based on the teaching of D1 and D2, the person skilled in the art would have a good expectation of success to arrive to the subject-matter of claims 1-8.

Specific embodiments such as the expression of recombinant oxalate oxidase enzyme in plants were known from D3: the "oxox" gene transferred in plants can be used to fight specific plant pathology (such as fungal disease in sunflower caused by sclerotinia, see D3 page 11). Thus, the vector description or RNA antisense strategy and the derived plant cells and plants mentioned in claims 2-21 merely can be considered as obvious embodiments or alternatives to a person skilled in the art. Therefore, in absence of surprising technical effects, no inventive step can be acknowledged for the claims 1-21.

In summary, the subject matter of claims 1-21 does not satisfy the criterion set forth in Article 33(3) PCT.

Item VIII**Certain observations on the international application**

1. The expression "...modified gene has a reduced tendency to silencing..." used in claim 1 is a vague. It corresponds to a relative definition without a clear reference point which therefore appears meaningless (what shall be considered as a "reduced tendency" and in comparison to what reference?). Therefore, claim 1 does not allow an unambiguous definition to the skilled person and results in lack of clarity (Article 6 PCT).
2. Claim 1 refers to the "actin 2 gene promoter". Such designation that is found throughout the claims is not suitable to clearly and unambiguously characterise a nucleic acid molecule. Relating to this, applicant's attention is drawn to the fact that a nucleic acid molecule is a chemical compound which can be clearly and unambiguously characterised by its nucleic acid sequence.
The same applies for the "gus gene" and "oxox gene" expressions used in claims 12,

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INTERNATIONAL PRELIMINARY

International application No. PCT/GB99/04317

EXAMINATION REPORT - SEPARATE SHEET

16 and 20.

3. The term "substantially the sequence shown in " in claim 3 is a relative definition open to interpretation, and thus render the scope of said claims unclear (Article 6 PCT).
4. Claims 5-7 and 14-15 refer to a RNA to be expressed in order to inhibit the production of a "homologous" protein. The term "homologous" embrace unspecified amino acid sequences (see also Reack GR et al, Cell 1987, Aug 28, 50(5):667), and thus render the scope of said claims unclear (Art. 6 PCT). The same remark applies for the "heterologous" protein mentioned in claims 6 and 13-15.

WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant in which the expression of the modified gene has a reduced tendency to silencing, which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.
2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.
3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.
4. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a heterologous protein.
5. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a homologous protein.
6. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant inhibits the production of a homologous protein.
7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.
8. A method as claimed in any of claims 1-7 in which the plant is lettuce or sunflower.

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell.

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11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein, or one conferring herbicide resistance.

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12. A plant cell as claimed in claim 11 in which the DNA construct is adapted to express the oxox gene.

13. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant homologous protein in the cell.

14. A plant cell as claimed in claim 9 adapted to express RNA that inhibits the production of a homologous protein.

15. A plant cell as claimed in claim 12 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

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16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

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17. *Compositae* plants comprising cells claimed in any of claims 9-15.

18. Plants as claimed in claim 17 which are lettuce.

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19. Plants as claimed in claim 17 which are sunflower.

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20. A plant claimed in any of claims 17-19 which is adapted to express the *oxox* gene and is resistant to sclerotinia.

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21. A plant claimed in any of claims 17-19 which is adapted to express a heterologous gene conferring herbicide resistance.

WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant in which the expression of the modified gene has a reduced tendency to silencing, which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.
2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.
3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.
4. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a heterologous protein.
5. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a homologous protein.
6. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant inhibits the production of a homologous protein.
7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.
8. A method as claimed in any of claims 1-7 in which the plant is lettuce or sunflower.

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.
10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell.
11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein, or one conferring herbicide resistance.
12. A plant cell as claimed in claim 11 in which the DNA construct is adapted to express the oxox gene.
13. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant homologous protein in the cell.
14. A plant cell as claimed in claim 9 adapted to express RNA that inhibits the production of a homologous protein.
15. A plant cell as claimed in claim 12 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.
16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

17. *Compositae* plants comprising cells claimed in any of claims 9-15.

18. Plants as claimed in claim 17 which are lettuce.

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19. Plants as claimed in claim 17 which are sunflower.

20. A plant claimed in any of claims 17-19 which is adapted to express the oxox gene and is resistant to sclerotinia.

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21. A plant claimed in any of claims 17-19 which is adapted to express a heterologous gene conferring herbicide resistance.

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22nd December 2000

European Patent Office
D-80298 Munich
GERMANY

Attention: Examiner O. Vix

Dear Sir,

PCT PATENT APPLICATION: PCT/GB99/04317
"GENETIC MODIFICATION OF COMPOSITAE"
APPLICANTS: ADVANTA TECHNOLOGY LTD et al.
Our reference: TR/RM/98102WO

Reference is made to the First Written Opinion dated 4 October 2000 drawn up by the International Preliminary Examining Authority.

NOVELTY

It is noted that no objection is taken to the claims on the ground of lack of novelty. Without considering the relevance of the intervening art D4, the Applicants submit that the original priority document clearly supports the claims on file.

INVENTIVE STEP

It is noted that claims 1-20 are considered to lack inventive step on the basis of the disclosure of D1 and D2.

In the view of the Applicants, this view is based on too general a view of the problem which the invention is designed to solve. The Examiner suggests that the problem is "a method for obtaining genetically-modified *Compositae* plant cells with an heterologous DNA construct under the control of a promoter capable of an effective expression in said plant". Since D1 (as acknowledged in the applicants' specification) discloses the plant promoter that applicants use, and D2 discloses both the promoter and a member of the class of plants to which the applicants' invention is applied, the invention is considered obvious.

However, Applicants contend that they have solved a more

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specific different problem. This is the problem of 'gene silencing'. It is known that *Compositae* can be genetically modified with heterologous DNA: however it is a recognised problem that after one or more generations the introduced gene is no longer expressed. Clearly it is of little use to modify a plant genetically by introducing a heterologous gene, if the character that the gene expresses is rapidly lost when the plant is reproduced. The present invention, by a suitable choice of one promoter among the many available, solves this problem.

If D2 is considered in this light, it is seen to be of little relevance. It is dealing with a quite different problem - that of making plants resistant to herbicides by inserting a particular transgene (protox). It discloses numerous promoters which may be used to activate the transgene (including the Actin 2 promoter) and numerous plant species that may be transformed by the transgene (including sunflower). But it gives no guidance to the person faced with the problem of how to overcome gene silencing in *Compositae* - it is quite silent on the advantages to be obtained by the use of the Actin 2 promoter. There is no teaching of the benefits that this specific promoter gives with either sunflower or lettuce. Accordingly, it is submitted, the present invention is in no way obvious.

The claims have been redrafted to bring this out more clearly. Basis for the amendment to claim 1 is to be found in the discussion at page 2 lines 11-18, setting out the problem, and at page 8 lines 6 to page 9 line 2, which shows that the present invention solves it. At the same time, a reference to herbicide resistance has been included in claim 11, and a new claim 21 to plants with herbicide resistance has been added.

OBSERVATIONS

1. Applicants respectfully disagree with the Examiner's comment. Expressions such as 'the actin 2 gene' or 'the oxox gene' are functional expressions that are well understood in the art. "The oxox gene" denotes a DNA sequence from which an enzyme having oxox functionality can be expressed. It is indeed true that a nucleic acid fragment can be characterised clearly and unambiguously by its nucleic acid sequence, just as a screw can be defined clearly and unambiguously in terms of its diameter, pitch and depth of groove. However, the purpose of a claim is not to characterise its elements in full structural detail: rather it is to define the Applicant's invention, in terms which will give him a fair scope of protection while affording the public reasonable certainty as to what is covered (EPC Article 69). Certainly, in a mechanical invention, a reference to a screw would not need to be supplemented by a specific recital of the dimensions of the screw used. To define inventions, functional

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expressions, as used here, are generally appropriate to define claim elements with reasonable generality.

2. The term 'substantially' has been deleted, though it is felt this term merely makes explicit what is always implicit in claim construction.

3. In the claims referred to, the terms 'homologous' and 'heterologous' are being used to refer to proteins produced by the organism from homologous (unmodified) DNA, and proteins produced by the organism from heterologous (transgenic) DNA. It is submitted that this terminology is quite clear in the context.

Yours faithfully,

A handwritten signature in cursive script, appearing to read "T W Roberts".

T W Roberts
Authorised Representative
Batchellor Kirk & Co

Enc: New set of claims (3 pages)

WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.
2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.
3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.
4. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a heterologous protein.
5. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a homologous protein.
6. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant inhibits the production of a homologous protein.
7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.
8. A method as claimed in any of claims 1-7 in which the plant is lettuce or sunflower.

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene
5 promoter.

10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell

10 11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein.

12. A plant cell as claimed in claim 11 in which the DNA construct is adapted to express the oxox gene.
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15 13. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant homologous protein in the cell.

20 14. A plant cell as claimed in claim 9 adapted to express RNA that inhibits the production of a homologous protein.

15. A plant cell as claimed in claim 12 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

25 16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the

actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

17. *Compositae* plants comprising cells claimed in any of claims 9-15.

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18. Plants as claimed in claim 17 which are lettuce.

19. Plants as claimed in claim 17 which are sunflower.

10 20. A plant claimed in any of claims 17-19 which is adapted to express the oxox gene and is resistant to sclerotinia.

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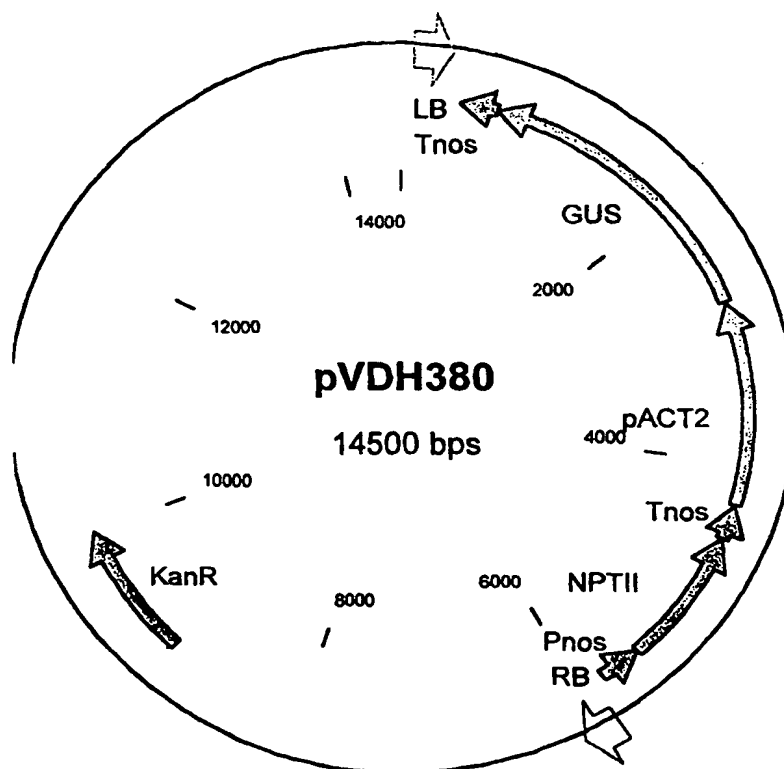
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(54) Title: GENETIC MODIFICATION OF *COMPOSITAE*

(57) Abstract

A method is disclosed of producing a genetically-modified *Compositae* plant. The plant is transformed with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.



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TITLE: GENETIC MODIFICATION OF *COMPOSITAE*

The present invention relates to the genetic modification of plants of the family *Compositae*, in particular lettuce (*Lactuca sativa*) and sunflower (*Helianthus annuus*).

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Genetic modification of plants is now well established as an experimental technique, and increasingly such plants are being used in agriculture in different parts of the world. The technique offers a number of advantages. The introduction of heterologous genes allows the plant to produce heterologous proteins with functions that the plant does not normally possess. For example, introduction of the gene for production of the *Bacillus thuringiensis* (Bt) insecticidal protein renders the plant toxic to insects and protects it against insect attack. Similarly genes may be introduced which protect against the attack of fungal and viral diseases. It is also possible by genetic modification to control the expression of homologous genes which the plant already possesses. Thus by inserting extra copies of homologous genes under the control of a suitable promoter, the expression of the protein produced by such genes can be up-regulated. Correspondingly, down-regulation may be induced by means such as anti-sense technology, in which an inverted copy of the homologous gene is inserted in the plant. Expression of the inverted gene produces antisense RNA, which inhibits the expression of the natural gene. In this way, for example, ripening of fruit such as tomatoes has been delayed by inhibiting the action of the polygalacturonase gene.

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The genetic modification of plants in this way offers many potential benefits to farmers, consumers and the environment. To farmers it offers the opportunity to avoid the labour and expense of chemical sprays; for consumers it can provide cheaper food of higher quality; it can protect the environment both by raising productivity (thereby

25

reducing pressure on agricultural land) and by reducing the amount of chemical pesticide introduced into the ecosphere.

A number of methods for transforming crop plants, both monocotyledons and
5 dicotyledons, are now well-known. They include, for example, the ballistic method (the
'gene gun') in which heavy metal pellets, for example of gold or tungsten, are coated
with DNA and fired into plant cells: and the Ti plasmid method. Another important
requirement for producing useful transformed plants is the availability of an effective
plant gene promoter. Many plant gene promoters are known: one very frequently used
10 constitutive promoter is the CaMV (cauliflower mosaic virus) 35-S promoter. However
not all such promoters are found to be equally effective in all plants. In some plants, in
particular *Compositae*, for example lettuce (*Lactuca sativa*) and sunflower (*Helianthus
annuus*), many heterologous constructs are found to have unstable expression levels.
Both in primary transformants and in progeny, 'gene silencing' often causes a severe
15 reduction in recombinant gene activity. In consequence, the plant reverts to a wild-type
phenotype. For practical applications of gene technology this is unacceptable, and
presents a real obstacle to the use of recombinant gene technology with plants such as
lettuce and sunflower.

20 According to the present invention, therefore, we provide a method of producing a
genetically-modified *Compositae* plant which comprises transforming the plant with a
heterologous DNA construct including a DNA sequence adapted to express RNA in the
plant under the control of the actin2 (ACT2) gene promoter. We further provide
genetically-modified *Compositae* plant cells comprising a heterologous DNA construct
25 including a DNA sequence adapted to express RNA in the plant under the control of the
actin2 (ACT2) gene promoter; and *Compositae*, in particular lettuce and sunflower,
plants comprising such cells.

The promoter of the actin2 (ACT2) gene derived from *Arabidopsis* has been shown to drive the beta-glucuronidase (GUS) gene in transgenic *Arabidopsis* plants to relatively high levels in vegetative tissues. In the inflorescence a developmental factor seemed to be involved causing a more differentiated expression pattern. This is reported by An, Meagher *et al.* (1996), *The Plant Journal* 10, 107-121: the same reference gives the DNA sequence of the actin-2 promoter (p.109).

The invention will be further described with reference to the drawings, in which:

Figure 1 is a map of a construct pVDH380 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive the beta-glucuronidase (GUS) gene.

Figure 2 is a map of a construct pVDH641 comprising the promoter of the actin2 (ACT2) gene derived from *Arabidopsis* arranged to drive the OXOX gene of wheat;

Figure 3 gives the DNA sequence of the actin2 (ACT2) promoter derived from *Arabidopsis*.

Actin is a fundamental cytoskeletal component essential to nearly all eukaryotic cells, in which it forms microfilament structures. There are large families of plant actin genes, with greater diversity than corresponding animal genes. The actin 2 gene promoter is a constitutive promoter to be found in most plants. We particularly prefer to use the actin 2 gene promoter obtained from *Arabidopsis thaliana*: though corresponding actin 2 promoters can readily be isolated from other sources, particularly other plants, and used for the same purpose.

The DNA sequence which expresses RNA may be of two main kinds: either a sequence which expresses mRNA which is translated into protein, or a sequence which

produces RNA which is not translated into protein, but which interacts with the biochemistry of the plant cell in another way, for example by inhibiting gene expression.

A preferred use of the present invention is to promote the expression of heterologous genes. However it may also be used to up-regulate or down-regulate the expression of homologous genes. As heterologous genes may be used DNA sequences coding for insecticidal proteins (for example the Bt protein) or fungicidal or antiviral proteins. ACT2 can be used to drive oxox (the oxalate oxidase gene), giving sclerotinia resistance, or other genes like early or late flowering genes (for example ATH1), herbicide resistance genes, insect tolerance genes (aphid resistance in lettuce), virus resistance genes (eg lettuce mosaic virus, LMV), nitrate reductase to lower nitrate content, and genes for increased shelf life. Many traits desirable in lettuce and sunflower can be exploited using this promoter.

The use of DNA sequences of homologous genes to inhibit or promote gene expression is quite well understood. A complete gene sequence, under the control of a suitable promoter, that operates effectively in the plant, will generally overexpress the gene product, leading to an amplification of the effect of the protein so produced. Sometimes the gene product is reduced: this phenomenon is termed "co-suppression". Reduction of the gene product is also generally obtained by reversing the orientation of the gene sequence with respect to the promoter so that it produces "antisense" messenger RNA.

A DNA construct for use in the invention may be an "antisense" construct generating "antisense" RNA or a "sense" construct (encoding at least part of the functional protein) generating "sense" RNA. "Antisense RNA" is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA:

complementary in the sense that each base (or the majority of bases) in the antisense sequence (read in the 3' to 5' sense) is capable of pairing with the corresponding base (G with C, A with U) in the mRNA sequence read in the 5' to 3' sense. Such antisense RNA may be produced in the cell by transformation with an appropriate DNA construct

5 arranged to generate a transcript with at least part of its sequence complementary to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). "Sense RNA" is an RNA sequence which is substantially homologous to at least part of the corresponding mRNA sequence. Such sense RNA may be produced in the cell by transformation with an appropriate DNA

10 construct arranged in the normal orientation so as to generate a transcript with a sequence identical to at least part of the coding strand of the relevant gene (or of a DNA sequence showing substantial homology therewith). Suitable sense constructs may be used to inhibit gene expression (as described in International Patent Publication WO91/08299) or a sense construct encoding and expressing a homologous functional

15 protein may be transformed into the plant to over-express the protein.

DNA constructs for use in the invention to inhibit gene expression may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA. There is no theoretical upper limit to the base sequence - it may be as long as

20 the relevant mRNA produced by the cell - but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used.

25

The invention will be further described with reference to the following Examples.

EXAMPLES

Lettuce was transformed with various constructs comprising the act2 gene promoter. The act2/GUS construct shown in Figure 1, kindly provided by courtesy of Dr. R. B. Meagher, Dept. of Genetics, University of Georgia, Athens, GA 30602, USA, and by us termed pVDH380 (Figure 1), was used to transform lettuce, in order to evaluate the expression pattern in primary transformants as well as the stability in consecutive generations. The construct pVDH380 was used to make a construct pVDH641 (Figure 2) containing the act2 promoter linked to the oxalate oxidase (OXOX) gene of wheat: this construct was used in transient expression studies in both lettuce and sunflower.

• Results

ACT2/GUS in lettuce

The binary vector pVDH380 of Figure 1 contains the NPTII gene as a selectable marker in addition to the ACT2/GUS gene. The act2 promoter sequence includes the first 19 codons of the act2 gene as well as the first exon-intron combination. Vector pVDH380 was used directly to transform lettuce (variety "Evola"), following the Ti plasmid method given in Curtis *et al.* (1994), J. Exp. Bot. **45**, 1441-1449.

From this transformation experiment were obtained a total of 38 independent transformants displaying a wide range of GUS-activities (as judged from a histochemical GUS staining using leaf explants of greenhouse grown material). These were compared with control CaMV 35S-GUS transformants, prepared similarly. The act2/GUS transformants of the invention showed higher and more uniform levels of GUS activity than the CaMV controls.

Subsequently, twelve independent act2/GUS transformants were used to carry out further histochemical assays. Tissues were taken from leaves, stems, roots, and flowers

(sepals, petals, stamens, carpels). The tissues examined showed consistent GUS activity levels for nine of the events. Three of the events however showed a certain degree of variability in expression (e.g. enhanced in flowers or vegetative tissues) which is probably due to the site of integration in the genome.

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It was concluded that the act2/GUS construct displays a relatively strong, predominantly constitutive expression pattern in transgenic lettuce at the T0 level.

Seeds were harvested and a total number of 15 events were analyzed in the T1 generation using greenhouse grown material. The segregation data in the T1 generation, as well as levels of GUS activity in the T0 generation, are shown in Table 1 below.

10

TABLE 1

Segregation analysis of transgenic lettuce expressing ACT2/GUS		
<i>Transformant code</i>	<i>GUS activity in T0</i>	<i>Segregation of GUS activity in T1</i>
Ev-1A-1	+	66+ / 17-
Ev-1A-3	++	20+ / 7-
Ev-2A-2	+	82+ / 24-
Ev-2A-4	-	0+ / 97-
Ev-4A-3	++	75+ / 22-
Ev-5A-2	-	0+ / 102-
Ev-5A-4	-	0+ / 97-
Ev-7A-1	-	0+ / 92-
Ev-8A-1	++	87+ / 22-
Ev-10A-1	+	75+ / 19-
Ev-10A-3	+	58+ / 20-
Ev-12A-1	+	36+ / 13-
Ev-13A-2	++	84+ / 3-
Ev-14A-1	++	78+ / 17-
Ev-14A-2	-	0+ / 98-

"+" = active; "++" = highly active; - = "not detected"

5

The most important observation is that there is no significant loss of GUS activity when the gene is transferred to the T1. This contrasts sharply with the situation in which

35S-GUS is used which typically results in a total inhibition of gene activity in 90% of the events during transmission from one generation to the next.

EXAMPLE 2

5 act2/OXOX in lettuce and sunflower

A further experiment was done to illustrate the use of the act2 promoter to drive other genes besides GUS. As the act2/GUS construct pVDH380 (Figure 1) contains 19 codons of the actin2 gene it was decided to modify the promoter by PCR using a primer combination which generates a unique restriction site at the act2 transcription start. This
10 modified promoter was fused to the OXOX gene and inserted into a binary vector, as described below.

Construction of ACT2-OXOX

Starting material for the ACT2-OXOX construct of Figure 2, termed pVDH641 (Figure 2), was the plasmid pVDH380 (Figure 1) which is identical with the plasmid
15 ACT2/GUS described by An et al., cited above.

Figure 1 shows a physical map of the construct pVDH380. In this figure, 'LB' indicates the left border, 'RB' indicates the right border, 'Pnos' indicates the nopaline synthase promoter, 'Tnos' indicates the nopaline synthase terminator, 'NPTII' indicates
20 the neomycin phosphotransferase II gene, 'pACT2' indicates the Actin 2 promoter, 'GUS' indicates the beta-glucuronidase gene and 'KanR' indicates the bacterial kanamycin resistance gene.

The ACT2 promoter was recloned from vector pVDH380 after amplification by PCR.

25

Figure 3 shows the nucleotide sequence of the Actin 2 promoter region. The sequence corresponding to the forward primer (bold, **Gothic typeface**) as well as to the

complementary sequence (bold underlined) of the backward primer are indicated. The start codon of the Actin 2 gene, ATG is given in bold capitals. In addition, the composition of the forward and backward primers are given. We used a primerset consisting of primer 1 (5'-GC AAGCTT ATT ATG ATC TCA AAT ACA TTG-3') and
5 primer 2 (5'-GC GGATCC TTT ATG AGC TGC AAA CAC AC-3'). Primer 1 contains after the first two nucleotides a HindIII restriction recognition site and subsequently a nucleotide sequence identical to the nucleotide sequence located from position 1358 to position 1379 upstream from the ATG-start codon (see Figure 3). Primer 2 contains after the first two nucleotides a BamHI restriction recognition site and subsequently 20
10 nucleotides complementary from position 3 to position 22 upstream from the start codon. The DNA fragment which was obtained after amplification was digested with HindIII and BamHI and inserted in the vector pVDH478. pVDH478 is a binary vector containing between the left and right border the NPTII gene, flanked upstream by the nopaline synthase promoter and downstream by the nopaline synthase poly(A)-signal. It also
15 contains the coding region of the oxalate oxidase gene (OxOx) with its own poly(A)-signal which is derived from wheat (for more information on the OxOx gene, including sequence data, see PCT Publication WO92/14824). The resulting vector was called pVDH641. A physical map of pVDH641 is shown in Figure 2. In this Figure, annotations in common with Figure 1 have the same meaning as in that Figure. Additionally,
20 'TOxOx' indicates the oxalate oxidase terminator, and 'OxOx' indicates the oxalate oxidase gene. The main restriction enzymes are indicated.

Sequence analysis confirmed that the ACT2 promoter region had been inserted without any mutation having occurred during the PCR amplification. OxOx activity can
25 be measured in a histochemical assay using oxalate which is converted by the enzyme into a purple dye. The act2/OXOX

fusion showed good levels of OxOx activity in transient assays, using both lettuce and sunflower explants, confirming the functionality of the construct.

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WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.
2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.
3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.
4. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a heterologous protein.
5. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a homologous protein.
6. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant inhibits the production of a homologous protein.
7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.
8. A method as claimed in any of claims 1-7 in which the plant is lettuce or sunflower.

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene
5 promoter.

10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell.

10 11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein.

12. A plant cell as claimed in claim 11 in which the DNA construct is adapted to express the oxox gene.
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13. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant homologous protein in the cell.

14. A plant cell as claimed in claim 9 adapted to express RNA that inhibits the
20 production of a homologous protein.

15. A plant cell as claimed in claim 12 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

25 16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the

actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

5

17. *Compositae* plants comprising cells claimed in any of claims 9-15.

18. Plants as claimed in claim 17 which are lettuce.

19. Plants as claimed in claim 17 which are sunflower.

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20. A plant claimed in any of claims 17-19 which is adapted to express the oxox gene and is resistant to sclerotinia.

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3 December 1999

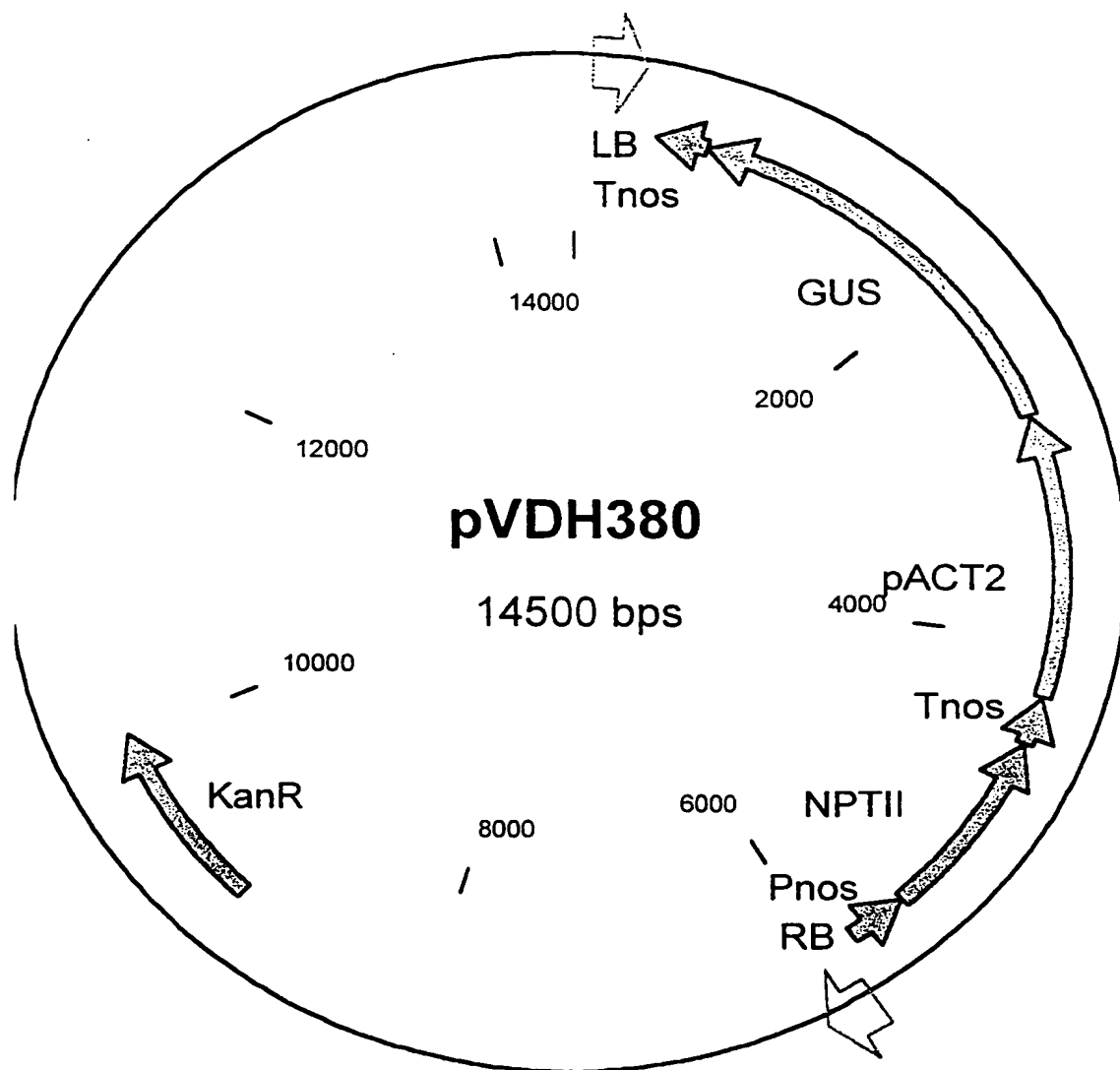
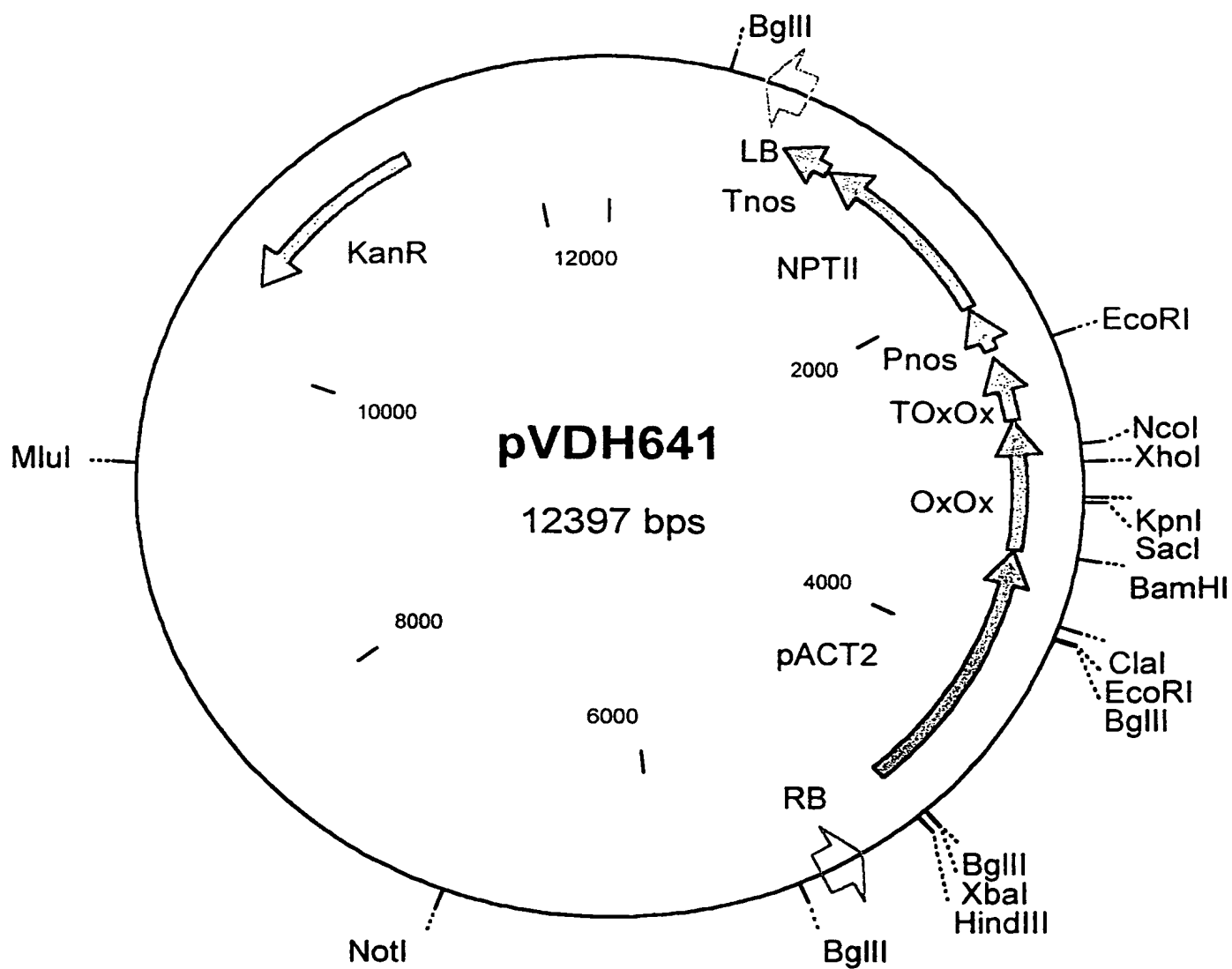


Figure 1

**Figure 2**

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1 **attatgatct caaatacatt** gatacatatc tcattctagat
 41 ctaggttatc attatgtaag aaagttttga cgaatatgnn
 81 acgacaaaat ggctacactc gatgtaattg gtatctcaac
 121 tcaacattat acttatacca aacattagtt agcaaaattt
 161 aaacaactat ttttatgtat gcaagagtca gcatatgtat
 201 aattgattca gaatcgtttt gacgagttcg gatgtagtag
 241 tagccattat ttaatgtaca tactaatcgt gaatagtgat
 281 atgatgaaac attgtatctt attgtataaa tatccataaa
 321 cacatcatga aagacacttt ctttcagggg ctgaattaat
 361 tatgatacaa ttctaataga aaacgaatta aattacgttg
 401 aattgtatga aatctaattg aacaagccaa ccacgacgag
 441 gactaacggt gcctggattg actcggttta agttaaccac
 481 taaaaaaacg gagctgtcat gtaacacgcg gatcgagcag
 521 gtcacagtca tgaagccatc aaagcaaaag aactaatcca
 561 aggggtgaga tgattaatta gtttaaaaat tagttaacac
 601 gagggaaaag ctgtctgaca gccaggtcac gttatcttta
 641 cctgtggtcg aaatgattcg tgtctgtcga ttttaattat
 681 ttttttgaaa ggccgaaaat aaagttgtaa gagataaacc
 721 cgcctatata aattcatata ttttcctccc cgctttgaat
 761 tgtctcgttg tcctcctcac tttcatcagc cgttttgaat
 801 ctccggcgac ttgacagaga agaacaagga agaagactaa
 841 gagagaaaag aagagataat ccaggagatt cattctccgt
 881 tttgaatctt cctcaatctc atcttcttct tccgctcttt
 921 ctttccaagg taataggaac tttctggatc tactttattt
 961 gctggatctc gatcttgttt tctcaatttc cttgagatct
 1001 ggaattcggt taatttggat ctgtgaacct ccactaaatc
 1041 ttttggtttt actagaatcg atctaagttg accgatcagt
 1081 tagctcgatt atagctacca gaatttggct tgaccttgat
 1121 ggagagatcc atgttcatgt tacctgggaa atgatttgta
 1161 tatgtgaatt gaaatctgaa ctgttgaagt tagattgaat
 1201 ctgaacactg tcaatgttag attgaatctg aacactgttt
 1241 aagtttagatg aagtttgtgt atagattctt cgaaacctta
 1281 ggatttgtag tgtcgtacgt tgaacagaaa gctatttctg
 1321 attcaatcag ggtttatttg actgtattga actctttttg
 1361 **tgtgtttgca gctcataaaa** aATGgctgag gctgacgata
 1401 ttcaaccaat cgtgtgtgac aatgggtactg gaatggtagg
 1441 atcc

HindIIIActin 2 primer forward. GC AAGCTT **attatgatct caaatacatt g****BamHI**Actin 2 primer backward GC GGATCC **tttatgagctgcaaacacac****Figure 3**

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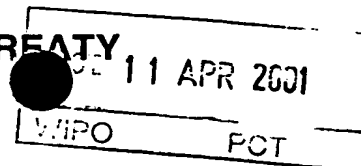
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Applicant's or agent's file reference DW/RM/98102WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/04317	International filing date (day/month/year) 16/12/1999	Priority date (day/month/year) 21/12/1998
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant ADVANTA TECHNOLOGY LIMITED et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 3 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the report</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input checked="" type="checkbox"/> Certain observations on the international application</p>		
Date of submission of the demand 16/06/2000	Date of completion of this report 09.04.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vix, O Telephone No. +49 89 2399 7326	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/04317

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-11 as originally filed

Claims, No.:

1-21 as received on 27/12/2000 with letter of 22/12/2000

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

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☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15,17-21
	No:	Claims	16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

- D1: AN YONG-QIANG ET AL: 'Strong, constitutive expression of the Arabidopsis ACT2/ACT8 actin subclass in vegetative tissues.' PLANT JOURNAL 1996, vol. 10, no. 1, 1996, pages 107-121, XP000876842 ISSN: 0960-7412 cited in the application
- D2: WO 97 32011 A (CIBA GEIGY AG ;VOLRATH SANDRA L (US); JOHNSON MARIE A (US); POTTER) 4 September 1997 (1997-09-04)
- D3: WO 92 14824 A (ICI PLC) 3 September 1992 (1992-09-03) cited in the application
- D4: WO 00 20571 A (SAAD MOHAMMED EID ;AGRICULTURAL GENETIC ENGINEERI (EG); PIONEER HI) 13 April 2000 (2000-04-13)

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The application relates to methods of producing a genetically modified Compositae plant by transformation of a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin 2 gene promoter from Arabidopsis thaliana.

1. Novelty (Art. 33(2) PCT)

- 1.1 Priority documents have not been available at the time of establishing this preliminary opinion. The opinion has been established under the assumption of valid priority rights. Should this however not be the case, the document D4 cited in the ISR as E-document might become important if its own priority date is valid.
- 1.2 D1 describes a vector comprising the ACT2 sequence fused with the beta-glucoronidase reporter gene (Gus) that is used to monitor the constitutive expression in different vegetative tissue(D1 page 111 and 119). As such, this construct in D1 takes away the novelty of claim 16 which relates to a vector including a Gus DNA sequence under the control of ACT2 gene promoter.

2. Inventive step (Art. 33(3) PCT)

Claims 1-8 refer to a method of producing a genetically-modified Compositae plant using an heterologous DNA sequence under the control of the actin 2 (ACT2) gene promoter. Claim 16 deals with the vector used in the process of claim 1.

The claims 9-15 are directed towards the genetically modified Compositae plant cells obtained by the process of claim 1, whereas claims 17-21 relate to the Compositae plants comprising those cells.

The closest prior art D2 discloses methods for engineering plants with DNA encoding protox (protoporphyrinogen oxidase) enzymes or mutants thereof. Examples of transgenic plants transformed with recombinant vectors (cited in D2 page 31) comprising protox gene coupled with an active promoter are disclosed in D2. The Arabidopsis actin promoter is clearly cited in D2 (page 37) as a promoter capable of functioning in plants or plant cells, clearly indicating to the skilled person a possible use of such a promoter in specific plant vectors. Moreover, diverse plants of agronomical interest are cited in page 32 of D2, and a Compositae plant (in this case sunflower) is cited among the most preferred plants. In consequence, a method for producing a transgenic sunflower using the transformation with a recombinant DNA vector comprising the actin promoter is clearly suggested in D2, although not disclosed specifically in the examples (choice in a list of plants and a list of possible promoters).

The problem to be solved by the present invention may therefore be regarded as a method for obtaining genetically-modified Compositae plant cells comprising a transformation of the plant with an heterologous DNA construct under the control of a promoter capable of an strong and effective expression in said plant.

From D1, it is known that the ACT2 gene promoter of Arabidopsis is a strong and constitutive actin gene promoter. In page 111 of D1, the construct comprising the ACT2 promoter coupled with the Gus reporter gene showed a strong expression in all vegetative tissues of the plant. Obviously the ACT2 promoter is an interesting candidate for strong recombinant expression in plants.

Therefore, the skilled person interested in solving the technical problem would combine the teaching of D2 (possibility to choose the sunflower for the transformation

using a recombinant DNA vector comprising a promoter such as Actin) with the technical features of D1 describing in details the technical features of the Actin promoter. Thus, based on the teaching of D1 and D2, the person skilled in the art would have a good expectation of success to arrive to the subject-matter of claims 1-8.

Specific embodiments such as the expression of recombinant oxalate oxidase enzyme in plants were known from D3 : the "oxox" gene transferred in plants can be used to fight specific plant pathology (such as fungal disease in sunflower caused by sclerotinia, see D3 page 11). Thus, the vector description or RNA antisense strategy and the derived plant cells and plants mentioned in claims 2-21 merely can be considered as obvious embodiments or alternatives to a person skilled in the art. Therefore, in absence of surprising technical effects, no inventive step can be acknowledged for the claims 1-21.

In summary, the subject matter of claims 1-21 does not satisfy the criterion set forth in Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The expression "...modified gene has a reduced tendency to silencing..." used in claim 1 is a vague. It corresponds to a relative definition without a clear reference point which therefore appears meaningless (what shall be considered as a "reduced tendency" and in comparison to what reference?). Therefore, claim 1 does not allow an unambiguous definition to the skilled person and results in lack of clarity (Article 6 PCT).
2. Claim 1 refers to the "actin 2 gene promoter". Such designation that is found throughout the claims is not suitable to clearly and unambiguously characterise a nucleic acid molecule. Relating to this, applicant's attention is drawn to the fact that a nucleic acid molecule is a chemical compound which can be clearly and unambiguously characterised by its nucleic acid sequence.
The same applies for the "gus gene" and "oxox gene" expressions used in claims 12,

16 and 20.

3. The term "substantially the sequence shown in " in claim 3 is a relative definition open to interpretation, and thus render the scope of said claims unclear (Article 6 PCT).
4. Claims 5-7 and 14-15 refer to a RNA to be expressed in order to inhibit the production of a "homologous" protein. The term "homologous" embrace unspecified amino acid sequences (see also Reeck GR et al, Cell 1987, Aug 28, 50(5):667), and thus render the scope of said claims unclear (Art. 6 PCT). The same remark applies for the "heterologous" protein mentioned in claims 6 and 13-15.

WE CLAIM:

1. A method of producing a genetically-modified *Compositae* plant in which the expression of the modified gene has a reduced tendency to silencing, which comprises transforming the plant with a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.
2. A method as claimed in claim 1 in which the ACT2 gene promoter is derived from *Arabidopsis thaliana*.
3. A method as claimed in claim 2 in which the ACT2 gene promoter has substantially the sequence shown in Figure 3.
4. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a heterologous protein.
5. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant codes for the production of a homologous protein.
6. A method as claimed in any of claims 1-3 in which the RNA to be expressed in the plant inhibits the production of a homologous protein.
7. A method as claimed in claim 6 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.
8. A method as claimed in any of claims 1-7 in which the plant is lettuce or sunflower.

9. Genetically-modified *Compositae* plant cells that may be produced by the process of claim 1 comprising a heterologous DNA construct including a DNA sequence adapted to express RNA in the plant under the control of the actin2 (ACT2) gene promoter.

10. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant heterologous protein in the cell.

11. A plant cell as claimed in claim 10 in which the heterologous protein is an insecticidal, fungicidal or antiviral protein, or one conferring herbicide resistance.

12. A plant cell as claimed in claim 11 in which the DNA construct is adapted to express the oxox gene.

13. A plant cell as claimed in claim 9 adapted to express RNA that produces recombinant homologous protein in the cell.

14 . A plant cell as claimed in claim 9 adapted to express RNA that inhibits the production of a homologous protein.

15. A plant cell as claimed in claim 12 in which the RNA to be expressed in the plant is antisense to DNA coding for a homologous protein.

16. A vector useful in the process of claim 1 which comprises a DNA construct including a DNA sequence adapted to express RNA in a plant under the control of the actin2 (ACT2) gene promoter, the DNA sequence comprising the gus gene or the oxox gene.

17. *Compositae* plants comprising cells claimed in any of claims 9-15.

18. Plants as claimed in claim 17 which are lettuce.

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19. Plants as claimed in claim 17 which are sunflower.

20. A plant claimed in any of claims 17-19 which is adapted to express the oxox gene and is resistant to sclerotinia.

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21. A plant claimed in any of claims 17-19 which is adapted to express a heterologous gene conferring herbicide resistance.

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PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

WEITZEL, David, S.
Brookes Batchellor
102-108 Clerkenwell Road
London EC1M 5SA
ROYAUME-UNIDate of mailing (day/month/year)
01 August 2001 (01.08.01)Applicant's or agent's file reference
021-2 (98102WO)International application No.
PCT/GB99/04317

IMPORTANT NOTIFICATION

International filing date (day/month/year)
16 December 1999 (16.12.99)

1. The following indications appeared on record concerning:

☐ the applicant
 ☐ the inventor
 ☒ the agent
 ☐ the common representative

Name and Address

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person
 ☐ the name
 ☒ the address
 ☐ the nationality
 ☐ the residence

Name and Address

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
 ☐ the International Searching Authority
 ☐ the International Preliminary Examining Authority
 ☐ the designated Offices concerned
 ☒ the elected Offices concerned
 ☐ other:
The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Form PCT/IB/306 (March 1994)

Authorized officer

R. Chrem

Telephone No.: (41-22) 338.83.38

004194562

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

02 August 2000 (02.08.00)

International application No.

PCT/GB99/04317

Applicant's or agent's file reference

021-2 (98102WO)

International filing date (day/month/year)

16 December 1999 (16.12.99)

Priority date (day/month/year)

21 December 1998 (21.12.98)

Applicant

VAN DUN, Cornelis, M., P. et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

16 June 2000 (16.06.00)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was



was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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